CHROMATOGRAPHIC ANALYSES OF AMINO ACIDS IN THE DEVELOPING SLIME MOLD, DICTYOSTELIUM DISCOIDEUM RAPER [1]

JEROME O. KRIVANEK AND ROBIN C. KRIVANEK

Department of Zoology, Newcomb College of Tulane University, New Orleans 18, Louisiana

The slime mold, *Dictyostelium discoideum* Raper, is a relatively simple biological system in which to study the processes of differentiation and morphogenesis. From a seemingly homogeneous mass of cells (the aggregation mass), there are eventually formed in the mature sorocarp two basic cell types—the stalk cell and the spore cell. The developmental cycle of *D. discoideum* has been described in detail by Bonner (1944) and Raper (1935, 1940) and will not be repeated here.

In the recent literature, studies have been reported that suggest correlations between nitrogen metabolism and the processes of differentiation and morphogenesis in this slime mold. Gregg, Hackney and Krivanek (1954) detected the evolution of ammonia and described changes in several nitrogenous fractions during the life cycle of this organism. In this same study, they suggested that the cellulose of the mature sorocarp was synthesized at the expense of a protein precursor and pointed out that the major nitrogen changes took place while the spore and stalk cells were being formed, *i.e.*, during the culmination process. In addition, Krivanek and Krivanek (1958), using the histochemical technique devised by Francis (1953), demonstrated the occurrence of amine oxidase activity in various regions of the slime mold undergoing differentiative changes. The simultaneous occurrence of changes in nitrogen metabolism and of differentiative and morphogenetic phenomena prompted the present study.

Materials and Methods

The method as outlined by Block, Durrum and Zweig (1955) was used for ascending paper chromatographic determinations of amino acids in the slime mold. Chromatograms, using hydrolyzed and unhydrolyzed tissues, were made of four representative stages of development—migrating pseudoplasmodium, pre-culmination, culmination, and mature sorocarp. In the case of hydrolyzed tissue, individuals in the desired stage of development were isolated and homogenized in 6 N HCl, hydrolyzed for 18 hours, and evaporated over a boiling water bath. The residue was placed in a soda lime desiccator for 48 hours and then taken up in 2 cc. of warm glass-distilled water and filtered. After evaporating the water filtrate, the residue therefrom was taken up in 1 cc. of iso-propanol, the vehicle used in the application of the spot. In the case of the unhydrolyzed tissue,

[1] This research was supported in part by Research Grant E 1453 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

homogenates were made with water and evaporated. The residue was taken up in 1 cc. of iso-propanol and applied to the paper.

The microhomogenizer described by Gregg, Hackney and Krivanek (1954) was used for the preparation of the tissue homogenates. All homogenization took place at room temperature (22° C.). Depending upon the stage of development to be analyzed, the homogenization procedure lasted from thirty minutes to an hour. All evaporation took place over a boiling water bath with the evaporation lasting

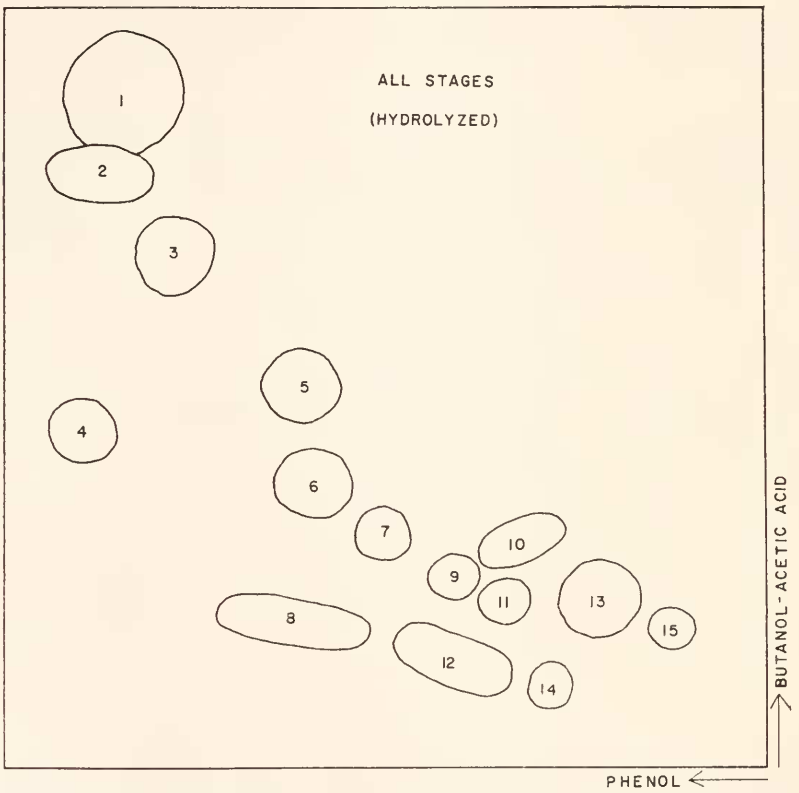

FIGURE 1. Diagram of the results of two-dimensional chromatography on hydrolyzed tissue of *D. discoideum*. Spots are identified as the leucines (1), phenylalanine (2), methionine (3), proline (4), tyrosine (5), alanine (6), threonine (7), histidine (8), glycine (9), glutamic acid (10), serine (11), asparagine (12), unknown (13), cystine (14), and aspartic acid (15).

no more than five minutes in any case. Rupture of virtually all cells was insured by means of periodic microscopic examination of the homogenate.

For both types of analyses, *i.e.*, hydrolyzed and unhydrolyzed, two-dimensional chromatograms were made on Whatman No. 1 filter paper. For the first dimension, n-butanol, acetic acid and water (250, 60, 250 v/v/v) were used as the solvent mixture. For the second dimension, an 80% solution of phenol in water was used as solvent. Development of the spots was accomplished by means of spraying the chromatograms with a solution of 0.3% ninhydrin in 95% ethanol. After

spraying, the chromatograms were allowed to dry in complete darkness for 18 hours. No less than 6 and no more than 10 runs were made for each analysis. In the majority of cases, consistent spot patterns were achieved and only 6 runs were made. However, in those few cases where slight inconsistencies in the patterns were evident, additional runs were made to achieve reproducibility.

Identification of the spots was achieved in two ways. Firstly, R_f values were calculated and compared with the R_f values of known amino acids. Secondly, one-dimensional as well as two-dimensional "control" runs were made using solutions of known amino acids, both singly and grouped, and the loci of spots were compared between the control and experimental series.

Results

Hydrolyzed tissue. Results of the chromatographic studies of amino acids in hydrolyzed tissues of *D. discoideum* are shown in Figure 1. With the exception

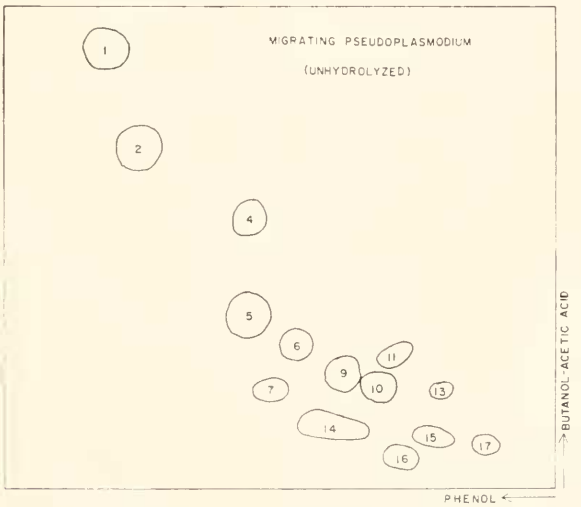

Figure 2. Diagram of the results of two-dimensional chromatography on unhydrolyzed tissue of *D. discoideum* in the migrating pseudoplasmodium stage. Identified spots are the leucines (1), methionine (2), tyrosine (4), alanine (5), threonine (6), glycine (9), serine (10), glutamic acid (11), aspartic acid (13), and cystine (15). Spots 7, 14, 16, and 17 are unknowns.

of one spot (no. 13), all spots were identified. The identified spots included the leucines (1), phenylalanine (2), methionine (3), proline (4), tyrosine (5), alanine (6), threonine (7), histidine (8), glycine (9), glutamic acid (10), serine (11), asparagine (12), cystine (14), and aspartic acid (15).

The same spot pattern persisted throughout the four analyzed stages of development. Although no quantitative determinations of the amino acids were made, comparisons of the relative spot intensities afforded some degree of quantification. Glutamic acid presented the most intense color in each stage. Also quite intense, but not to the degree of glutamic acid, were the spots of the leucines, methionine, alanine, threonine, serine, and asparagine. Medium light spots resulted from

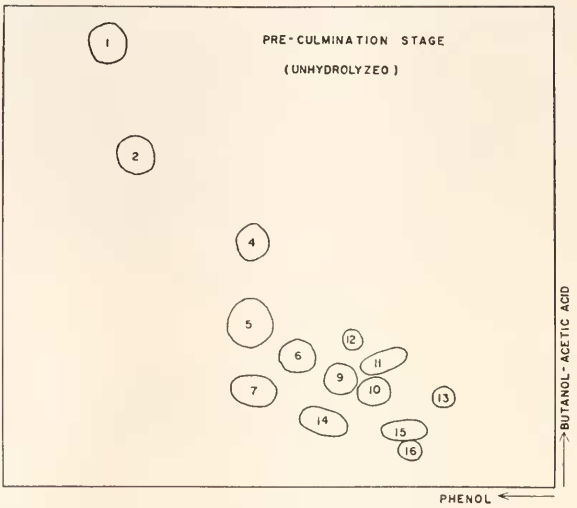

FIGURE 3. Diagram of the results of two-dimensional chromatography on unhydrolyzed tissue of *D. discoideum* in the pre-culmination stage. Spots as in Figure 2, plus spot 12, an unknown.

phenylalanine, tyrosine, glycine, and histidine. The faintest spots were those of proline, cystine and aspartic acid.

In addition to these well-formed spots, a very faint, vaguely-defined spot was occasionally found in the approximate locus of cysteine. Because of its vagueness

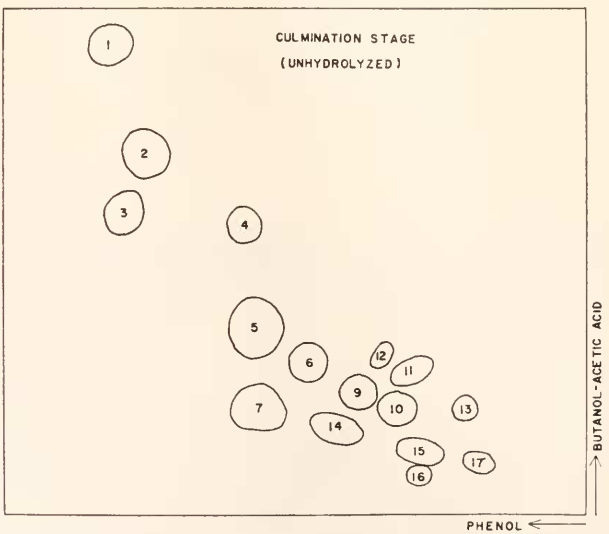

FIGURE 4. Diagram of the results of two-dimensional chromatography on unhydrolyzed tissue of *D. discoideum* in the culmination stage. Spots as in Figure 2, plus spots 3 and 12, unknowns.

and the failure of our controls to show a clear cysteine spot, we cannot state positively either the presence or absence of cysteine.

Unhydrolyzed tissue. Results of the chromatographic studies of amino acids in unhydrolyzed tissue of *D. discoideum* are shown in Figures 2, 3, 4 and 5. Whereas a consistent spot pattern occurred throughout the developmental cycle in the case of hydrolyzed tissue, considerable variability in the spot patterns occurred between the several stages in the case of unhydrolyzed tissue. A total of 17 spots appeared in all or nearly all of the stages of development. However, only ten were identified. They were the spots of the leucines (1), methionine (2), tyrosine (4), alanine (5), threonine (6), glycine (9), serine (10), glutamic acid (11), aspartic acid (13), and cystine (15). The remaining seven spots—3, 7, 8, 12, 14, 16, and 17—were not identified. Presumably these ninhydrin-positive

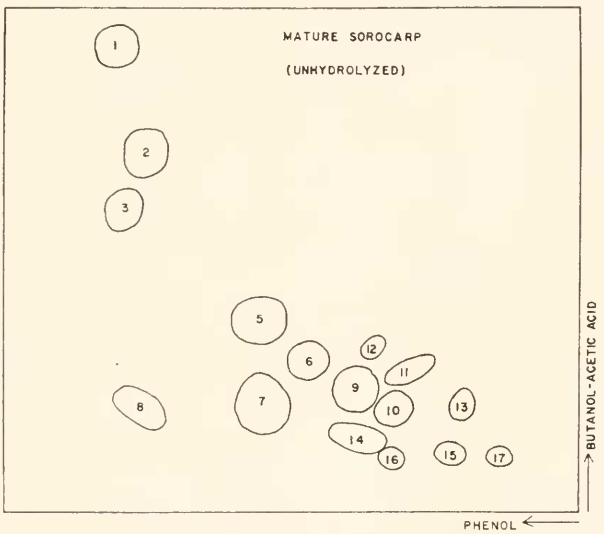

FIGURE 5. Diagram of the results of two-dimensional chromatography on unhydrolyzed tissue of *D. discoidcum* in the mature sorocarp stage. Spots as in Figure 2, plus spots 3, 8, and 12, unknowns.

spots were simple peptides. It is possible that these spots were the products of partial hydrolysis by enzymes derived from the cells. However, in view of the rapidity with which the tissues were prepared, this would seem unlikely. Those spots which were evident in all stages of development were 1, 2, 5, 6, 7, 9, 10, 11, 13, 14, 15, and 16. Although spot 12, an unknown, did not appear in the migrating pseudoplasmodium, it did appear in the succeeding three stages of development. Spot 3, also unknown, appeared only in the culmination and mature sorocarp stages, while spot 8, a third unknown, appeared only in the mature sorocarp stage. Spot 4, identified as tyrosine, was present in all stages except the mature sorocarp, and spot 17 appeared erratically—being present in all but the pre-culmination stage. As in the case of hydrolyzed tissue, cysteine could not be definitely ascertained as either being present or absent.

Discussion

In their quantitative studies of the nitrogen metabolism in the slime mold, *D. discoideum,* Gregg, Hackney and Krivanek (1954) demonstrated a decrease in the total nitrogen/dry weight during the transition from the migrating pseudoplasmodium to the mature sorocarp. They attributed this decrease to a decrease in the total extractable protein nitrogen and total unextractable nitrogen components of the slime mold. In addition, they found that ammonia was being given off by the slime mold during its life cycle. In a subsequent study, Gregg and Bronsweig (1956) found a steady increase in the total amount of reducing substances (presumably carbohydrates) as the life cycle progressed. On the basis of these data, it was suggested that the protein fraction of the slime mold served as a precursor for the carbohydrate of the mature sorocarp. However, no indication was made of the possible pathway(s) involved in this conversion. The present study may be suggestive in this respect.

Glutamic acid invariably presented the most intense spot of any of the determined amino acids. This was evident in both hydrolyzed and unhydrolyzed tissues. The deamination of glutamic acid to α-ketoglutaric acid with the corresponding release of ammonia is known. Because of the reversibility of this reaction, it is considered to be one of the prime mechanisms responsible for the interconversion of ammonia and α-amino group nitrogen. The reaction is catalyzed by glutamic acid dehydrogenase, requiring either DPN or TPN as a coenzyme (Meister, 1957). The importance of this reaction, as it relates to *D. discoideum,* lies in the fact that not only has glutamic acid been detected to a high degree in the slime mold, but, also, the liberation of ammonia during the life cycle suggests such a deamination reaction. Further, Krivanek and Krivanek (1958) demonstrated non-specific dehydrogenase activity in the pre-stalk area of the pre-culmination and culmination stages—stages in which the future sorophore sheath (consisting primarily of polysaccharides) is being secreted by the stalk cells as they move apically to become eventually enclosed within the sorophore sheath. This non-specific dehydrogenase activity could logically be attributed to glutamic acid dehydrogenase. By virtue of the relationship between glutamic acid, α-ketoglutarate, areas of dehydrogenase activity, and sites of carbohydrate secretion, there thus can be postulated this link between carbohydrate metabolism and protein metabolism in the slime mold.

The glutamic acid-ketoglutarate relationship, if actually operative in the slime mold, need not be the only link between carbohydrate metabolism and protein metabolism. Aspartic acid, also demonstrated in hydrolyzed and unhydrolyzed tissues of the slime mold, can be deaminated to fumarate, another intermediate in the citric acid cycle (Meister, 1957), thus creating a second possible link between the two types of metabolism. Further, there is the possibility that alanine can undergo deamination forming the Krebs cycle intermediate—pyruvate—as has been suggested by Meister (1957), and serine, as well as cysteine, can undergo the same process yielding ammonia and pyruvate.

The suggested relationships already discussed do not preclude the possibility of other mechanisms relating carbohydrate metabolism to protein metabolism, such as decarboxylation and transamination. There is as yet, however, no evidence to indicate the presence of these mechanisms in the slime mold.

Several of the amino acids of the hydrolyzed tissues appear as well in unhydrolyzed tissue. Consequently, it is not possible to determine whether these amino acids occur as free amino acids only, or also as bound amino acids. However, four amino acids appear only in the hydrolyzed tissue (phenylalanine, proline, histidine, and asparagine). They are considered therefore to exist only in the bound form. The significance of these amino acids with respect to the differentiative process in *Dictyostelium* is at present not apparent.

Studies have recently been initiated to test the validity of the above postulates. These correlative studies will embrace the use of the analogs of the amino acids shown to be present in *D. discoideum.*

Summary

1. The amino acids in hydrolyzed and unhydrolyzed tissue of the slime mold, *Dictyostelium discoideum* Raper, have been determined by means of two-dimensional ascending paper chromatography. Analyses were made on four stages of development—migrating pseudoplasmodium, pre-culmination, culmination, and mature sorocarp.

2. Unhydrolyzed tissue contained the leucines, methionine, tyrosine, alanine, threonine, glycine, serine, glutamic acid, aspartic acid, cystine, and seven unidentified spots, presumably simple peptides. Not all these spots were present in all tested stages.

3. Hydrolyzed tissue contained in addition to the amino acids identified above, phenylalanine, proline, histidine, asparagine, and one unknown spot. All tested stages were identical.

4. The postulate is presented that glutamic acid (and possibly also to a lesser degree aspartic acid, alanine, serine, and cysteine) through deamination may enter the Krebs cycle and form a link between protein and carbohydrate metabolism, the change in balance between protein and carbohydrate being one of the most prominent features of differentiation in this organism.

LITERATURE CITED

Block, R. J., E. L. Durrum and G. Zweig, 1955. A Manual of Paper Chromatography and Paper Electrophoresis. Academic Press, Inc., New York.

Bonner, J. T., 1944. A descriptive study of the slime mold, *Dictyostelium discoideum. Amer. J. Bot.,* **31**: 175–182.

Francis, C. M., 1953. Histochemical demonstration of amine oxidase in liver. *Nature,* **171**: 701–702.

Gregg, J. H., A. L. Hackney and J. O. Krivanek, 1954. Nitrogen metabolism of the slime mold *Distyostelium discoideum* during growth and morphogenesis. *Biol. Bull.,* **107**: 226–235.

Gregg, J. H., and R. D. Bronsweig, 1956. Biochemical events accompanying stalk formation in the slime mold, *Distyostelium discoideum. J. Cell. Comp. Physiol.,* **48**: 293–300.

Krivanek, J. O., and R. C. Krivanek, 1958. The histochemical localization of certain biochemical intermediates and enzymes in the developing slime mold, *Dictyostelium discoideum* Raper. *J. Exp. Zool.,* **37**: 89–116.

Meister, A., 1957. Biochemistry of the Amino Acids. Academic Press, Inc., New York.

Raper, K. B., 1935. *Dictyostelium discoideum,* a new species of slime mold from decaying forest leaves. *J. Agric. Res.,* **50**: 135–147.

Raper, K. B., 1940. Pseudoplasmodium formation and organization in *Dictyostelium discoideum. J. Elisha Mitchell Sci. Soc.,* **56**: 241–282.